

DNA Cleanup Kit

Aim of the experiment

The protocol is used to further purify and clean the DNA obtained from phages for later analysis.

Materials

- Phage solution from Page T7 DNA Purification Protocol
- Norgen Biotek-Corp.Kit (Kit-Cat.:46800)
 - Lysis Buffer B
 - Wash Solution A
- RNase free DNase I (NEB DNase I (RNase-free) #M0303L)
- Proteinkinase (Proteinkinase K from NEB #P8107S)
- 15 ml Falcon Tubes
- 1.5 ml microcentrifuge tubes
- Nuclease-free H₂O

Procedure

1. DNA isolation as described in "Page T7 DNA Purification"-Protocol until step 10.
2. Transfer Phage solution in a 15 ml Tube.
3. DNase treatment: Add 10 µl of RNase-Free DNase I (20 Units) to phage solutions.
4. Incubate for 15 minutes at RT.
5. Inactivate DNase I at 75°C for 5 minutes.
6. Transfer phage solution to 1.5 ml centrifuge tube.
7. Add 500 µl of Lysis Buffer B
8. Vortex solution for 10 seconds.
9. Add 4 µl (20 mg/ml) Proteinkinase K and incubate at 55°C for 30 minutes.
10. Incubate at 65°C for 15 minutes.
11. Invert the lysate 2 – 3 times during the 15 minutes incubation time.
12. Add 320 µl isopropanol and vortex.
13. Prepare Spin columns and collecting tubes.
14. Apply 650 µl lysate to the column.

15. Centrifuge at 8,000 rpm (6,000 g) for 1 minute.
16. Discard the flowthrough.
17. Repeat step 14 – 16 until the entire lysate has passed through the column.
18. Add 400µl of Wash Solution A to column.
19. Centrifuge at 8,000 rpm (6,000 g) for 1 minute.
20. Discard the Flowthrough.
21. Repeat step 18 – 20 two more times.
22. Spin the (dry) column for 2 minutes at 14,000 rpm.
23. Place the column into a fresh 1.5 ml centrifuge tube.
24. Add 50 – 75 µl nf H₂O.
25. Centrifuge for 1 minute at 8,000 rpm.
26. Nanodrop to get DNA concentration.
27. Samples with a concentration between 40-90 ng/µl have been concentration up to 150 ng/µl.
28. Prepare agarose gel: 1% Agarose gel, gRed Staining, 1kb Extended Ladder.